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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

DEC 30 1992

Memorandum:

SUBJECT: 037100-ER. Diflubenzuron Metabolism in the Goat. P-chloroaniline (PCA) levels in milk and liver. (CBTS# 10828, MRID#'s 417021-02, 420609-01, 424942-01, -02, and -03, Barcode#D184050).

FROM: Jerry B. Stokes, Chemist
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THRU: Debra Edwards, Chief
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Debra Edwards 12/30/92

TO: P. Hutton/P. Schroeder, Pm #18
Insecticide-Rodenticide Branch
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and

Toxicology Branch
Health Effects Division (H7509C)

This is an expedited review at the request of RD. The Diflubenzuron Registration Standard Update stated that additional data is needed in regard to the possible presence of p-chloroaniline and its precursors containing the chlorophenylamine moiety (p-chlorophenyl urea and p-chloroacetanilide) in animals as a result of in vivo metabolism of diflubenzuron.

In response, the petitioner, Solvay Duphar B.V., The Netherlands, has submitted the following goat metabolic study (MRID#424942-01) entitled,

"Characterization of Residues of 14C-Diflubenzuron in Lactating Goat,

Volume I of III: Metabolite Patterns,

Volume II of III: A Method for the Determination of Trace Levels Of 4- Chloroaniline in Goat Liver: Development, Validation, and Application,

Volume III of III: A HPLC Method for the Determination of 4-Chloroacetanilide, 4- Chloroaniline, and Diflubenzuron in Goat Liver and Milk".

The petitioner has also previously submitted a method for the analysis of PCA in milk (See memo of 9/6/91, S. Malak), and the specific analyses of the milk samples from the above goat study entitled,

"A Method for the Determination of Trace Levels Of 4-Chloroaniline in Goat Milk: Development, Validation, and Application". (MRID#417021-02).

The petitioner has also previously submitted the biological report for this goat metabolism (See memo of 3/16/92, J. Stokes) entitled,

"The Disposition of 14C-Diflubenzuron in the Lactating Goat". (MRID#420609-01).

Detailed Considerations

Four female goats weighing 33-49 kg were orally dosed twice daily for 3 consecutive days with 14C-diflubenzuron, uniformly labelled in both phenyl rings, either at a high dose, 2.5 mg/kg body weight [batch AOI88K02A, sp. act., 19.5 uCi/mg, radiochemical purity, >99% (chemical purity is undefined)(goats #1 and #3)] or, a low dose, 0.1 mg/kg body weight [batch AOI88K03A, sp. act., 1.0 uCi/mg, radiochemical purity, >99% (chemical purity is undefined) (goats #4 and #5)]. Diflubenzuron reference standard, batch AOI85E08A, had 99.8% chemical purity and 99.3% radiochemical purity, and batch ARS85J30N had chemical purity >99.5% (radiochemical not defined).

The low dosage represents an average daily feeding level of approximately 10 ppm based on an assumed feed and hay consumption of 1 kg/day. The high dosage represents an average daily feeding level of approximately 250 ppm based on an assumed feed and hay consumption of 1 kg/day. Samples of milk, urine, and feces were collected twice daily and at 15 hours after the last dose and stored frozen until analysis. One control goat was used in the study; milk, urine, and feces were collected on the same schedule as the treated goats. Within 15 hours after the last dose, the animals were sacrificed and the tissues, organs, and the remaining carcass were analyzed for total 14C-activity.

Aliquots of each milk, urine, and bile sample were analyzed for total 14C-activity by LSC. All tissues, organs, and feces of the treated goats were homogenized, combusted, and the released $14CO_2$

trapped and measured by LSC. Radiochromatograms were constructed to plot the net dpm for each HPLC fraction versus time.

The administered 14C-dosage was excreted in the urine at an average of 12% and 6%, in the feces at an average of 77% and 81%, and in the intestinal tract contents at an average of 8 and 15%, respectively, for the low and high doses. From the urine, feces, and intestinal tract, the total 14C-recovery was 97% and 102% for the low and high dose, respectively. Uptake of residues was low for all tissues and organs. In the low dose treatment the highest 14C-residue level was in the liver at 0.8% TRR, while milk gave 0.1% TRR, and kidney, spleen, and bile were 0.01 % TRR. In the high dose treatment the highest 14C-residue level was in the liver at 0.6% TRR), while milk gave 0.09% TRR (residues appeared to plateau at or soon after day 1); kidney gave 0.02 % TRR; spleen and bile showed no 14C-activity above background (30 dpm). In both doses the remaining carcass gave 0.5% TRR.

TOTAL RADIOACTIVITY (14C-DIFLUBENZURON EQUIVALENTS) IN ORGANS, TISSUES, AND BODY FLUIDS IN PPM

Sample	10 ppm dose		250 ppm dose	
	ANIMAL 1	ANIMAL 3	ANIMAL 4	ANIMAL 5
Milk	0.009	0.007	0.22	0.22
Liver	0.217	0.262	3.24	6.06
Kidney	0.019	0.016	0.36	1.02
Spleen	0.002	0.002	0.05	0.07
Fat	0.003	0.004	0.12	0.30
Muscle	ND	0.001	0.02	0.05
Bile	0.151	0.229	20.75	2.59
Intestines (Contents/Walls)	0.214	0.187	4.41	9.53
Carcass	0.006	0.008	0.12	0.18

IDENTIFICATION OF 14C-RESIDUES IN MILK AND LIVER

The character of the 14C-residues in liver and milk was analyzed using two different HPLC systems for both. The HPLC profiles showed that the liver residue was comprised of more than 15 peaks, ranging from 0.002 to 0.033 ppm (low dose), and from 0.029 to 0.91 ppm (high dose). Milk consisted of 8 peaks. Enzymatic digestion to release any bound 14C-residues did not significantly alter the HPLC profiles. Enzymatic deconjugation of milk showed the percentage of sulfate and glucose conjugates is estimated at 20%. This same hydrolysis of liver did not yield deconjugation products. Based upon the high extraction recovery percentages of the total 14C-activity in liver (90%) and milk (85%), there is no evidence of bound residues of concern in liver or milk.

Unknown residues were compared with the following reference standards:

DFBU:	2,6-diflubenzuron
DFBA:	2,6-difluorobenzoic acid
DFBAM:	2,6-difluorobenzamide
DFHA:	2,6-difluorohippuric acid
CPU:	4-chlorophenylurea
PCA:	4-chloroaniline
PCAA:	4-chloroacetanilide
2HDFBU:	2-hydroxydiflubenzuron
3HDFBU:	3-hydroxydiflubenzuron
NA4CPU	N-acetyl-4-chlorophenylurea
4CNB:	4-chloronitrobenzene

LIVER

The HPLC solvent system I was a reverse phase step-wise gradient separation starting with a mobile phase of 65% dioxane: water (9:1), 35% acetonitrile, and ending with 35% dioxane:water, 65% acetonitrile on a C-8 column. Retention times are given for the reference standards DFBAM, CPU, PCAA, PCA, and DFBU. The major portion of the collected radioactivity eluted in the DFBAM fractions (55-77%). A smaller amount eluted with a retention similar to CPU (9-16%). No PCA or PCAA were detected using this solvent system for either the low or high dose. A limit of 30 dpm above background is used in the data summary for most results. At quite low levels of 14C activity, a limit of 10 dpm is used. Based upon the 30 dpm limit, the estimated limit of detection for PCA and PCAA was 0.001 ppm. However, a reasonable limit of quantification would be 0.005 ppm.

The HPLC solvent system II was a reverse phase step-wise gradient separation starting with a mobile phase of 100% diammonium hydrogenphosphate (3 g/l water), and ending with 90% methanol on a C-8 column. Retention times are given for the reference metabolite

standards DFBU, DFBA, DFBAM, DFHA, CPU, PCA, 2HDFBU, 3HDFBU, NA4CPU, and 4CNB; PCAA was not reported on this column. The major portion of the collected radioactivity eluted in the CPU/PCA fractions (11-16%). Smaller amounts eluted with retention times similar to DFBAM and DFBU (1.5-5% and 3.5-7%, respectively). The remaining radioactivity was spread over the profile. The highest percentage of other peaks was 12% (at approximate retention time of 2HDFBU); all others were less 10%. Small levels of radioactivity were located in the elution times for DFBAM, and parent DFBU. Metabolites CPU and DFBAM have been found in the rat metabolism. As discussed above, the estimated limit of detection was 0.001 ppm; the limit of quantification would be approximately 0.005 ppm.

Since PCA eluted in the same retention time as CPU on the C-8 column with this mobile phase, the presence of PCA was determined by a gas chromatography method equipped with EC detection (Volume II of III: A Method for the Determination of Trace Levels Of 4-Chloroaniline in Goat Liver: Development, Validation, and Application). Validation of this method was performed on both spiked samples from the control goat #2 and blank. To determine if DFBU, PCAA, or CPU could interfere with the extraction and/or analysis of PCA these compounds were also added to the spiked goat liver samples and quality control blanks. The limit of quantification for PCA for this glc method is 0.005 ppm. Previously the limit of quantification for the 14C-treated goat was 0.01 ppm. Recovery of PCA from the spiked samples averaged 109%; spiked samples freeze-stored for 22 months gave 106% recovery. Using this methodology, the concentration of PCA in the low dosed goats is below the limit of quantification of 0.005 ppm. In the high dosed goats the levels of PCA were 0.011 ppm (goat #4) and 0.028 ppm (goat #5).

In summary, the extraction of the liver yielded 90% of the total liver 14C-activity. Cleanup and analysis of this extract by HPLC gave >70% recovery of the 14C-activity of the liver. Of this percentage, approximately 31% had retention times similar to reference standards, i.e., DFBAM (1%), 2HDFBU (7%), CPU (16%), and DFBU (7%), by HPLC solvent system II. The small residue amounts prevented confirmation by other techniques. The remaining activity eluted over the run with small peaks, most <5% of the applied amount, and only one at 11%. None of these other HPLC peaks coincided with HPLC retention times of any reference standard.
In a previously submitted cattle ruminant metabolic study at 250 ppm diet for 8 days, the total of 6 ppm 14C-diflubenzuron equivalents found in the liver was defined by TLC as DFBU (14%), DFBA (51%), PCA (5%), CPU (<1%), and 29% as unidentified components (See MRID#00070185). This translates to approximately 0.3 ppm of residues of p-chloroaniline in the 250 ppm diet, similar to the 150 ppm diet used in this goat study. Therefore, in the expected daily dietary exposure of 9 ppm (based upon the existing uses including

the bolus, plus the proposed citrus use), levels of residues of p-chloroaniline would be ca. 0.001 ppm, below the limit of quantification (0.005 ppm).

The petitioner also submitted an additional HPLC analysis of PCAA, CPU, and DFBU in milk and liver (Volume II of III: "A HPLC Method for the Determination of 4-Chloroacetanilide and Diflubenzuron in Goat Liver and Milk") for the freeze-stored spiked samples from control goat #2 and quantity control blanks. The method gave recoveries for the liver of 97% for CPU, 123% for PCAA, and 100% for DFBU, and for milk of 90% for CPU, 101% for PCAA and 88% for DFBU. The limit of quantification in liver is 0.02 ppm for CPU, 0.05 ppm for PCAA, and 0.05 ppm for DFBU. In milk, the limit of quantification is 0.03 ppm for CPU, PCAA, and DFBU. This method uses a mobile phase and other separation conditions slightly different from those reported above in the HPLC systems I and II. The method is adequate for the levels of quantification stated.

MILK

Both HPLC solvent systems I and II were used to analyze milk for the low and high dosed goats. System I gave only elution of measurable residues in the elution areas for DFBAM and CPU (68-86% of total eluting 14C) for both dose levels; PCA was not detected. In the low dosed goats, system II showed the possible presence of CPU (29-42% total eluting 14C) and DFBAM (4-8% total eluting 14C). The remaining radioactivity was spread over the profile. All other peaks were <10% of the total eluting 14C. In the high dosed goats, system II showed the possible presence of CPU (32-54% total eluting 14C) and DFBAM (3-7% total eluting 14C). The remaining radioactivity was spread over the profile. All other peaks were <10% of the total eluting 14C. The estimated limit of detection for PCA and PCAA was 0.001 ppm; the limit of quantification would be approximately 0.005 ppm.

Since CPU and PCA elute at the same retention time in system II, the samples were also analyzed by an independent glc method. PCA was determined in milk by the same glc-EC method as used for the analysis of the liver. Validation of this method was performed on both spiked samples from the control goat #2 and blank. DFBU, PCAA, or CPU did not interfere with the extraction and/or analysis of PCA when these compounds were added to the spiked goat milk samples and quality control blanks. The limit of quantification for PCA for this glc method is 0.001 ppm. Recovery of PCA from the spiked samples averaged 84% (0.001 to 0.005 ppm); spiked samples (0.002 ppm) freeze-stored for 22 months gave an average recovery of 67%. Using this methodology, the concentration of PCA in the milk of both the low and high dosed goats is below the limit of quantification of 0.001 ppm.

In summary, the extraction of the milk yielded 85% of the total milk 14C-activity. Cleanup and analysis of this extract by HPLC

gave >70% recovery of the 14C-activity of the milk. Of this percentage, most had retention times similar to reference standards, i.e., DFBAM (6-8%) and CPU (29-55%) by HPLC solvent system II. The small residue amounts prevented confirmation by other techniques. There is no PCA reported at or above the level of quantification in milk (0.001 ppm). The remaining activity eluted over the run with small peaks, all <10%. None of these other HPLC peaks coincided with HPLC retention times of any reference standard.

Conclusion/Recommendation:

At the estimated daily dietary exposure of ca. 9 ppm diflubenzuron (based upon the existing uses including the bolus, plus the proposed citrus use), the concentration of PCA in ruminant milk is below the limit of quantification of 0.001 ppm. In the liver the maximum levels of PCA expected based upon this 9.0 ppm daily dietary exposure would be below the limit of quantification of 0.005 ppm.

A maximum level of 0.03 ppm CPU (p-chlorophenyl urea) could be in ruminant liver based on this 9.0 ppm exposure. Also based on the data CPU could be present in milk at 0.003 ppm.

If present, PCAA (p-chloroacetanilide) in liver and in ruminant milk would be below the limit of detection of 0.001 ppm.

The petitioner stated the following in regard to the purity of the 14C-diflubenzuron: "The presence of small traces of PCA as impurity in the radiolabelled diflubenzuron batches used for dosage cannot be excluded. Based upon radiochemical purity measurements, ≤ 0.1-0.4% PCA may be present in radiolabelled diflubenzuron (high and low specific activity, respectively). Determination of the PCA levels in radiolabelled diflubenzuron with the aid of the GC-ECD analytical method indicates a maximum presence of 0.05-0.2% PCA. This may, however, (have) resulted from diflubenzuron during work-up procedure. PCA found in liver after this diflubenzuron administration to lactating goats may, therefore, be partially due to the presence of this impurity." Based upon the submitted data PCA was not present in milk (<0.001 ppm) or in liver (<0.01 ppm).

Note: The petitioner has not submitted analysis data of the diflubenzuron batches to support this argument. Although CBTS does not have this data, we can agree with the fact that degradation of diflubenzuron during the sample preparation can theoretically account for small traces of PCA. Traces of PCA in the 14C-diflubenzuron used in this metabolic study should not affect the results of the study. CBTS does request the registrant to submit the chemical analysis of the 14C-diflubenzuron (batches AOI88K02A and AOI88K03A) used in this metabolic study.

Also, **at this time**, TOX is not concerned about the very low levels of residues with the 2,6-difluorophenyl moiety, i.e., DFBA and DFBAM (Private communication, H. Spencer, 12/17/92).

Therefore, based upon the results of this study, **and the registrant verifying a high chemical purity of the labelled diflubenzuron**, the metabolism data requirement in ruminants is now satisfied. Also based on the results of this study and the previously submitted ruminant study (MRID#00070185), and TOX considerations permitting, the residue of concern in meat and meat byproducts of cattle, swine, horse, goat, sheep, and milk, is the parent only, diflubenzuron.

NOTE: CBTS can only recommend for the proposed citrus tolerance (PP#1F2507) provided the petitioner submit the requested chemical purity data for review and CBTS determines this data to be adequate.

cc: PP#1F2507; J. Stokes (CBTS); H. Spencer (TOX); E. Zager (CBRS); diflubenzuron S.F.; R.F.; Circu
RDI: PErrico:12/17/92:RLoranger:12/30/92
H7509C:CBTS:JStokes:js:Rm 803:CM#2:305-7561:12/30/92